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This review focuses on the thermodynamic basis of the unfavorable changes observed in physicochemical properties in lead discovery and optimization programs and suggests that monitoring binding thermodynamics could contribute to an improvement in the quality of compounds identified.

Thermodynamics guided lead discovery and optimization

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The documented unfavorable changes of physicochemical properties during lead discovery and optimization prompted us to investigate the present practice of medicinal chemistry optimization from a thermodynamic perspective. Basic principles of binding thermodynamics suggest that discriminating between enthalpy-driven and entropy-driven optimizations could be beneficial. We hypothesize that entropy-driven optimizations might be responsible for the undesirable trend observed in physicochemical properties. Consequently, we suggest that enthalpy-driven optimizations are preferred because they provide better quality compounds. Monitoring binding thermodynamics during optimization programs initiated from thermodynamically characterized hits or leads, therefore, could improve the success of discovery programs. Here, we summarize common industry practices for tackling optimization challenges and review how the assessment of binding thermodynamics could support medicinal chemistry efforts.

Introduction

More than a decade ago, Teague *et al.* [1] investigated the physicochemical profile of screening compounds and concluded that polar and low molecular weight (MW) starting points were more easily converted to leads than lipophilic and higher MW hits. They proposed that a suitable screening library should consist of compounds with a MW range between 100 and 350 and clogP = 1–3. It was suggested that hits from such lead-like libraries would provide a wider chemistry space during the optimization of potency, physicochemical and absorption, distribution, metabolism and excretion (ADME) properties. The effect of lead optimization on physicochemical properties has been analyzed in comparative studies between leads and corresponding drugs [1,2]. These studies demonstrated that leads are typically less complex (with lower MW, fewer rings and rotatable bonds) and less hydrophobic (lower clogP) than drugs and suggest that the lead optimization process results in more complex structures. Although there might be notable differences in the physicochemical profile of compounds optimized against different target families, Morphy showed [3] that property shifts associated with optimization vary only slightly across target families. Thus, we can conclude that lead optimization is a major contributor to the unfavorable change in properties of clinical candidates.

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Attempts to control property shifts

MW and lipophilicity have a major impact on compound quality because – in addition to physicochemical properties – these parameters have a considerable impact on drug metabolism and pharmacokinetics (DMPK) and safety profile. MW increases in parallel with complexity, yielding large and complex molecules that are more likely to form suboptimal or repulsive interactions upon binding to proteins, as demonstrated by Hann *et al.* [4]. Moreover, highly lipophilic compounds have a greater chance of being promiscuous [5,6]; they typically have limited solubility and ADME/DMPK problems [7]. Because these properties have a direct impact on clinical success rates [8], two strategic improvements have been introduced to the practice of lead optimization. First, compounds prepared in optimization programs are now screened extensively in physicochemical and *in vitro* ADME assays to evaluate their property profile. Second, following the original idea of Teague *et al.* [1], it was concluded that less complex, polar, low MW hits serve as better starting points for optimization. Fragment-based drug discovery (FBDD) straightforwardly realizes this concept. Restricted size and complexity of fragments results in low binding affinity; therefore, identification of fragment hits requires high concentration screening and, consequently, high solubility for fragments. The fragment space defined by the 'rule of three' [9] typically fulfills these criteria. FBDD reviews frequently claim that physical properties could be more easily controlled in optimizing fragments than starting from higher affinity high throughput screening (HTS) hits (see, for example, Ref. [10]). The hope that unfavorable property shifts could be avoided by using FBDD strategies has generated considerable interest in the medicinal chemistry community. Investigating whether fragments could really help reduce property shifts, we collected fragment hit-lead pairs from the literature [11–14] and compared their physicochemical profiles to those of recent HTS hits and HTS leads. Our hit-to-lead database consists of 59 HTS [15] and 34 fragment hits and leads, respectively, which were all screened against the same set of targets, including proteases, kinases and GPCRs (Supplementary Data).

HTS hits typically showed micromolar affinity with a mean pPotency of 6.01 (Fig. 1a), which was higher than the pPotency

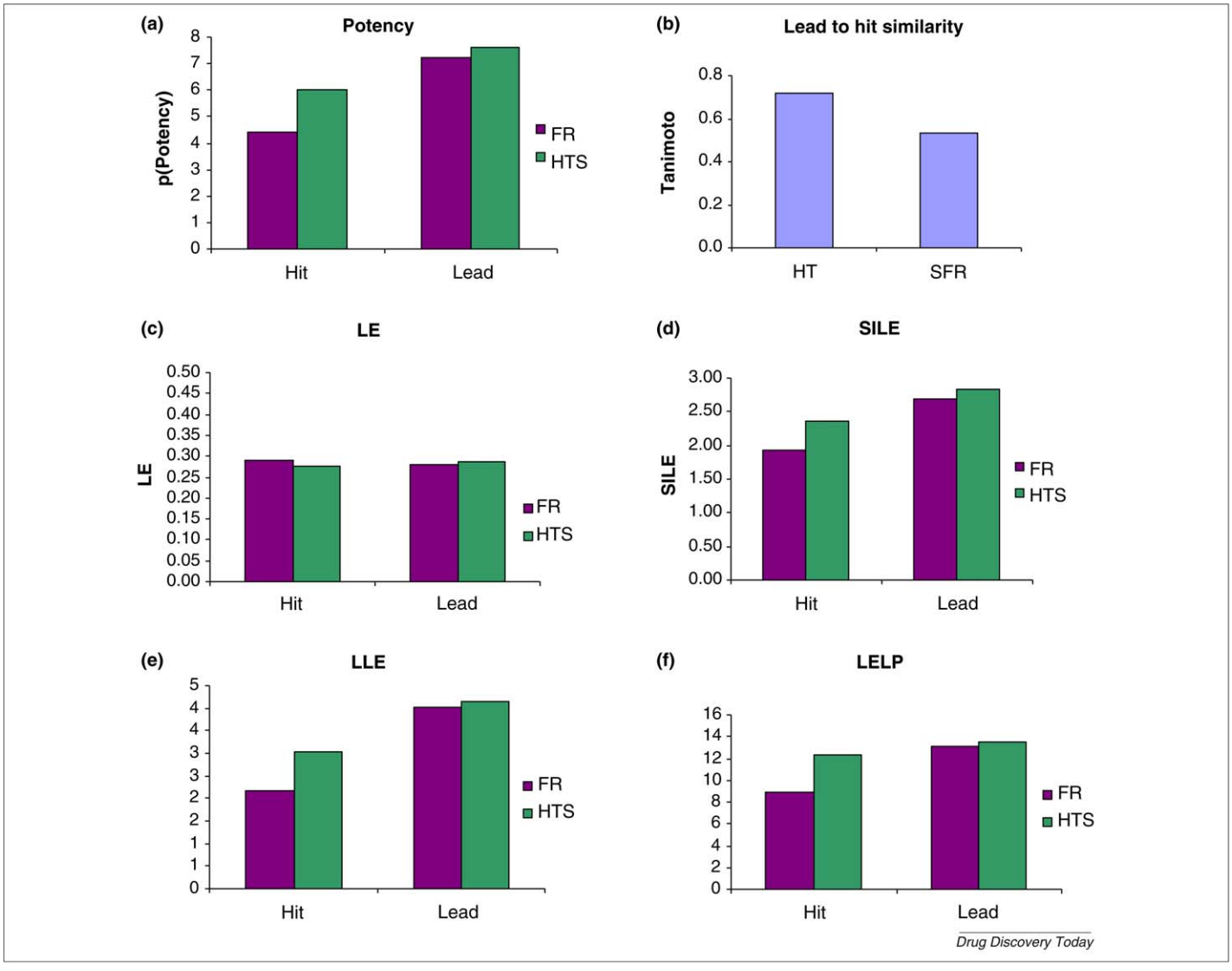


FIGURE 1

Average properties of fragment (FR) and HTS hits and leads.

obtained for less complex fragment hits (pPotency = 4.41), as expected. Hit-to-lead optimization from HTS hits resulted in HTS leads with a median pPotency of 7.60, representing an increase in affinity of approximately one order of magnitude. In the case of fragment hits, the mean pPotency of leads reached 7.20, revealing that fragment hits could be effectively optimized to leads having similar affinity to that of HTS leads. In addition to the significant increase in affinity, the average of Tanimoto similarities calculated between corresponding hit and lead pairs (Fig. 1b) suggests that hit-to-lead optimization of fragment hits led to structurally dissimilar leads. The larger chemistry space and the increased freedom of operation associated with fragment hits seem to be reflected in Tanimoto indices obtained for fragment- (0.53) and HTS-based (0.72) optimizations.

Next, we investigated ligand efficiency measures, including original ligand efficiency (LE) [16] and size-independent ligand efficiency (SILE) [17]. Contrary to the frequently cited phenomena of the high LE of fragments [10], we found that HTS hits and leads have LE similar to that of fragment pairs (Fig. 1c). It seems that the high potency of HTS hits compensates for their more complex nature, resulting in high LE for those HTS hits that could be followed up and optimized to leads. It was interesting to see, however, that the initial LEs did not improve for either fragment- or HTS-based optimizations. Because the size dependency of LE became obvious recently, we also compared SILE for fragment and HTS hits and leads (Fig. 1d). SILE of HTS hits and leads was higher than that of the fragments, although their difference became marginal for leads. Comparing lipophilic efficiencies, we found that the better lipophylic ligand efficiency (LLE) [15] of fragment hits disappeared for leads having virtually identical LLE to that of HTS leads (Fig. 1e). Recently, we introduced a new metric – LELP, defined as the ratio of logP and LE [15] – to depict the price of LE paid in logP (i.e. a lower absolute value of LELP is better). The present analysis revealed that LELP does not improve during the optimization of HTS hits to leads. More importantly, increasing LELP values associated with hit-to-lead optimizations of fragments suggests that improved affinity of fragment leads was primarily achieved by adding lipophilicity (Fig. 1f). Although LELP indicates that lipophilic efficiency deteriorates during hit-to-lead optimizations, SILE values demonstrate that ligand efficiency improves for both fragment and HTS hits and, furthermore, that this improvement seems to be more significant for fragment hits. The significant increase in affinity combined with large structural changes during hit-to-lead optimization underlines the advantage of fragment-based approaches over conventional HTS-based optimization because the former provides diverse lead chemotypes with almost the same affinity for leads as HTS leads. Although these advantages made fragment-based approaches popular in early-phase discovery, analyzing the basic properties of fragment hits and leads highlighted that, like HTS hits, the properties of fragments are also shifted unfavorably (Fig. 2).

At the hit-identification phase, fragment hits are notably less complex and lipophilic than HTS hits, as described in most case studies reporting fragment-based hit discovery [11–14]. This is one of the conceptual advantages of fragment screening: we can pick up a less complex and more soluble starting point for hit-to-lead studies. Unfortunately, our most important finding is that these good-quality fragment hits are optimized to leads with high MW and logP. Contrary to previous hopes of fragment-based approaches, this observation suggests that maintaining or improving LE or SILE alone is not a guarantee for high-quality leads. Fragment leads have a MW almost identical to that of HTS leads, and both groups have a similarly high logP that is in line with present medicinal chemistry practice [5]. Although most of the HTS-based optimizations do start from hits of higher MW and lipophilicity, this seems to be under control, as suggested by average changes detected during their optimization (ΔMW 75.63, ΔlogP 0.49). Fragment optimization, however, increased MW and lipophilicity more significantly (ΔMW 173.33, ΔlogP 0.93) as compared to their HTS-based counterparts, indicating that efficient optimization of fragments to potent leads is challenging (i.e. physicochemical properties could not be controlled easily, despite the attractive initial properties). These data indicate that present medicinal chemistry practice optimizes both HTS and fragment hits to leads that end up in the center of drug-like space. Consequently, further optimization with the same practice would shift the resulting candidate to the edge of the Lipinski zone with suboptimal physicochemical properties.

The impact of lead optimization on undesired property changes has been well documented. Analysis of a large number of hit and lead pairs identified by different strategies, including high-throughput screening, fragment screening, natural product

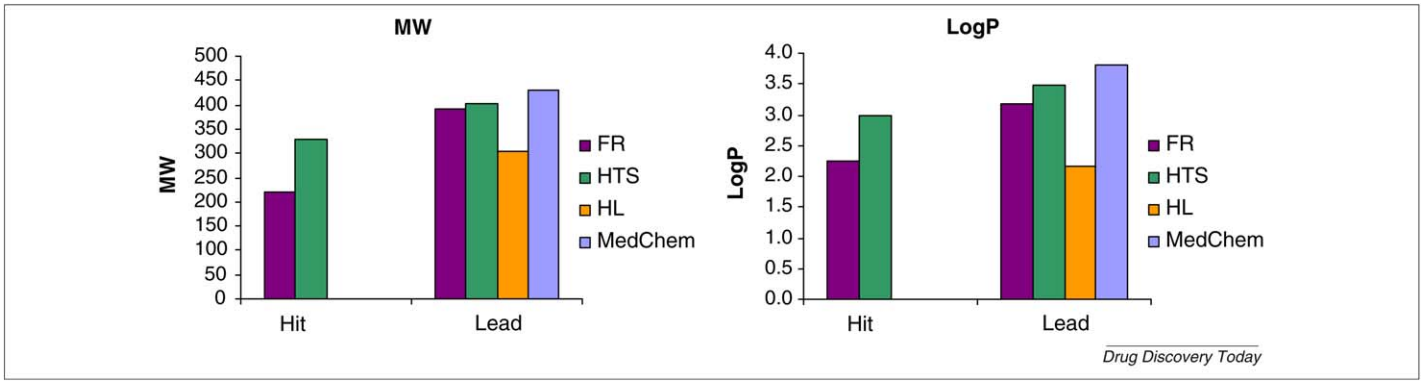


FIGURE 2

Median molecular weights and logP calculated for fragment (FR) and HTS hits and leads. Corresponding values of historical leads (HL) [15] and compounds representing the present medicinal chemistry practice (MedChem) [5] are depicted for comparison.

screening and virtual screening, has demonstrated [15,18] that this unfavorable shift in physicochemical properties can be traced back to lead discovery. We concluded that the increase in logP and MW during hit-to-lead optimization is independent of the nature of the library screened, the detection technology applied and the lead discovery strategy used. Here, we show that fragment-based approaches cannot avoid unfavorable property shifts *per se*. These observations suggest that it is the optimization practice that is a major contributor to property shifts, which prompted us to investigate the thermodynamic basis of optimization.

Thermodynamics of optimization

The most important objective of hit or lead optimizations is improving ligand binding. The logarithm of binding affinity – usually quantified by K_d or K_i values – is proportional to the Gibbs binding free energy (ΔG_{bind}, Eq. (1)).

$$\Delta G_{bind} = RT \ln K_d \sim RT \ln K_i \quad (1)$$

where R is the gas constant, T is the absolute temperature, K_d is the apparent equilibrium dissociation constant and K_i is the inhibition constant defined by the Cheng–Prusoff equation [19]. ΔG is a function of the binding enthalpy (ΔH) and the binding entropy (ΔS).

$$\Delta G_{bind} = \Delta H_{bind} - T\Delta S_{bind} \quad (2)$$

From a thermodynamic point of view, Eq. (2) suggests that the real challenge of medicinal chemistry optimization is to overcome enthalpy–entropy compensation [20]. There are two alternatives to achieve this goal: enthalpy-driven optimizations are characterized by decreasing ΔH that dominates over disfavored ΔS changes, and entropy-driven optimizations could be realized by increasing ΔS to compensate for ΔH penalties.

Ligand binding is a multistep process that involves the conformational rearrangement and desolvation of both the ligand and the binding site and that is followed by the formation of the ligand–receptor complex. Assuming equilibrium thermodynamics, each of these elemental steps contributes to the binding thermodynamics of the ligand. Ligand binding is usually accompanied by conformational rearrangement of both the ligand and the receptor, and this typically represents an enthalpic penalty. Desolvation restructures organized water clusters around the ligand, which results in a significant entropic reward. Replacement of water from the binding site might be both enthalpic and entropic, depending on the binding interactions of the replaced waters. H-bonds broken upon desolvation, however, are responsible for an additional enthalpic penalty. Formation of the ligand–receptor complex is typically coupled to forming new interactions between the ligand and its binding site that are enthalpically beneficial. Molecular recognition of the ligand, however, limits its external rotational and translational freedom (as well as ligand and protein flexibility) and, therefore, represents an entropic penalty. Although the thermodynamic impact of long-range effects is usually neglected, they could also contribute to ligand binding. The net effect of these enthalpy and entropy components determines whether the binding is enthalpy or entropy dominated; thus, the optimization can be enthalpy or entropy driven, depending on which component contributes more significantly to the affinity improvement.

Considering the enthalpic and entropic components of ligand binding, it was concluded that enthalpy-driven optimization is challenging [21]. Significant gain in binding enthalpy is associated with the formation of new contacts with optimal geometry that require new interaction partners such as charged groups, donors and/or acceptors at the ligand side. These new heteroatoms disfavor desolvation of the ligand and result in an enthalpic penalty. Because the new interactions formed upon binding reduce ligand and protein flexibility, they also contribute to the decrease of conformational entropy. Consequently, the gain in binding enthalpy could be easily compensated by enthalpic and entropic penalties caused by disfavored changes in desolvation and conformational entropy. In the case of entropy-driven optimizations, the gain in binding entropy could be realized by the increased lipophilicity of the ligand. More lipophilic compounds desolvate more easily, resulting in a significant reward in desolvation entropy. In addition, medicinal chemistry efforts reducing ligand flexibility – which usually increase MW and complexity – decrease the penalty arising from conformational entropy changes. Significant gain in desolvation entropy in conjunction with decreased penalty from conformational entropy is hardly compensated by enthalpic penalties. Because the optimization of specific interactions is far more difficult than increasing lipophilicity and complexity, entropy-driven optimization [21] seems to be a straightforward approach for medicinal chemistry teams working with strict timelines. In fact, entropy-driven optimization by adding lipophilic moieties and applying chain-ring strategies are successful tools routinely used in medicinal chemistry programs. Most of these optimizations, therefore, have significant entropy components, giving a thermodynamic rationale for undesirable property shifts. A recent article by Ladbury *et al.* [22] supports this hypothesis. The authors analyzed more than 400 isothermal calorimetry data obtained on more than 250 protein–ligand complexes and found a correlation between binding free energy and apolar surface burial upon complex formation. This finding is in accordance with the general medicinal chemistry observation that lipophilic interactions have a crucial role in binding affinity. Although the correlation between $-T\Delta S$ and apolar surface burial was less remarkable, they identified a statistically significant trend indicating that increasing apolar surface burial is entropically favored. This result gives additional support to the notion that entropic optimization would be a major source of increasing lipophilicity and complexity documented in the medicinal chemistry literature.

Guidelines for thermodynamics-driven optimization

Resolution of the binding free energy into entropy and enthalpy components goes beyond the usual characterization by affinity and it might be found useful at various stages of the hit to drug candidate process.

A major challenge in optimization is that it is easier to achieve improved binding by increased hydrophobicity than by optimized polar interactions. Although it is a general assumption that improved polar interactions lead to more favorable binding enthalpy, we cannot fully control the enthalpy by simply engineering interactions. For this reason, it is advantageous to measure the binding free energy components at an early stage of drug discovery to guarantee advantageous polar interactions and to monitor them in the course of optimization. At decision points, such as hit or lead selection, the enthalpy content of binding is an

important piece of information to consider when one compares the potential of compound series (i.e. to assess whether an affinity gain together with a favorable physicochemical profile can be achieved upon optimization). In this respect, thermodynamic signature shows similarity to metrics that define LE and, in particular, to LLE [5] and LELP [15]. Compound characterization by LLE and LELP aims to support the optimization of affinity without increasing lipophilicity. Importantly, compound polarity does not necessarily correlate directly to the enthalpic component of binding. It is not the presence of the polar groups but their favorable interactions with the protein that contribute to the increase of binding enthalpy. This explains why no correlation was observed between the binding enthalpy and polarity-related Lipinski parameters for oral bioavailability [20]. The presence of polar groups is, however, a prerequisite for high binding enthalpy.

A measure of the enthalpic content of the binding is enthalpic efficiency (EE) [23], which is defined as $\Delta H/N_{hv}$, where N_{hv} is the number of non-hydrogen atoms. (An alternative called 'specific EE' and defined as $\Delta H/N_{polar}$, with N_{polar} being the number of polar atoms, has also been proposed [23].) EE is similar to LE, but ΔH in EE replaces pK_i in LE. It is well documented, however, that LE depends on the number of heavy atoms [17,24] and the SILE was defined as $pK_i/N_{hv}^{0.3}$ [17]. SILE enables an unbiased comparison of ligands of different sizes. Concerning EE, we have shown elsewhere [25] that it strongly depends on the number of atoms and that this dependence is different from that found for LE. The maximal observed ΔH increases (i.e. becomes less favorable) with increasing atom number and so does the maximal EE (EE_{max}). Nevertheless, the trend between N_{hv} and EE_{max} agrees with that found between N_{hv} and LE_{max} [17], although the parameters are different. The size dependency of EE would, therefore, potentially mislead chemists when compounds based on different scaffolds and sizes are compared. To avoid such size-biased prioritizations, we introduced [25] the size-independent enthalpic efficiency (SIHE), defined as $SIHE = 0.018 \times \Delta H \times N^{0.3}$ if ΔH is obtained at 300 K and is expressed in kcal/mol units. SIHE is a meaningful measure of the optimization potential of compounds helping series selection and optimization monitoring before late optimization.

An optimal scenario of the optimization achieves affinity increase with only a modest growth in MW and lipophilicity. This can be realized by increasing the enthalpy content of the binding via the introduction of optimized polar interactions. Unfortunately, engineering of such interactions is a challenging task for several reasons. Polar interactions are highly sensitive to the relative positions of the interacting partners; in most cases, this sensitivity exceeds the precision our predictive tools can reach. Furthermore, the additivity of the free energy or its components is an approximation that also represents a hurdle in compound design [26]. Although it is reasonable to assume the additivity of the enthalpy of pairwise non-bonded interactions, the same cannot generally be assumed for the entropy and the free energy. This is due to the new or lost specific interactions that will change the number and population of available states for the system [27]. This issue is also related to the ubiquitous phenomenon of enthalpy–entropy compensation when, for example, the creation of a strong H-bond results in a favorable enthalpy gain that is largely compensated by an unfavorable entropy loss caused by the decrease of the available states for the system. These limitations of ligand design call for the experimental monitoring of the optimization by both structural studies and binding enthalpy measurements. Whereas X-ray or nuclear magnetic resonance (NMR) structures give a basically qualitative picture of the ligand–protein binding, thermodynamic data enable a quantification of the interactions. Thermodynamic data can come from the measurement of K_d at different temperatures followed by the application of the van't Hoff equation to derive ΔH and $T\Delta S$ [28] or from isothermal titration calorimetry (ITC) experiments [29,30]. Both of the techniques provide the net thermodynamics of ligand binding that makes the structural interpretation of these data challenging. The former approach requires that enthalpy shows no temperature dependence ($\Delta C_p \sim 0$), which is less typical for systems other than membrane proteins [31]. The ability to spot curvature in the plot caused by experimental error in K_d further complicates deriving thermodynamic parameters by the van't Hoff equation. Steady-state measurements over a broad temperature range and rigorous curvature analysis, therefore, are suggested to obtain confident datasets. Finally, interdependency of ΔH and $T\Delta S$ impacts the interpretation of enthalpic and entropic components of ligand binding. ITC has the advantage of measuring ΔH directly, but its principal limitations are high protein requirement and low throughput. It should be noted that enthalpy values can change dramatically depending upon such conditions as temperature, pH, buffer, and so on. On one hand, enthalpy data should be corrected for superimposed protonation steps [26] and ion binding and release [32] if necessary. On the other hand, because enthalpy is an integral function of the heat capacity change, it might be important to measure ΔC_p as well. Recent efforts with enthalpy arrays and automated ITC instruments promise to alleviate limitations in throughput [33,34]. Considering all of the limitations associated with the experimental evaluation of binding thermodynamics, carefully checked data generated for a series of compounds in unified conditions (a case typical in pharma optimizations) can be directly compared and analyzed.

In most cases, quantitative structure–activity relationships (QSAR) use K_d or IC_{50}, which are directly related to ΔG. A beneficial alternative is ΔH [35,36] because it better reflects the interactions between the ligand and the target. With accurate ΔH values made available by ITC, this avenue can readily be explored. Although ΔH is not the ultimate function we might want to optimize, a quantitative determination of ΔH enables us to have a better understanding of the interactions and to control the enthalpy content of binding.

Although thermodynamic and structural studies are mutually corroborating and are best used together, in cases in which structural information is not available, thermodynamic experiments can provide us with information on the binding interactions and thus also with quantitative experimental feedback on the success of compound design.

The thermodynamic analysis of ligand–receptor binding can, in some cases, also provide us with information on the agonist or antagonist nature of ligands. Agonists and antagonists can bind to the same receptor with different thermodynamic signatures. After the pioneering work of Weiland *et al.* [37], it turned out that the relative enthalpy and entropy components of agonist versus

antagonist binding are receptor dependent [38–42]. Furthermore, it was demonstrated that the discrimination depends on the experimental conditions applied [43,44]. This could suggest that the interactions of agonists and antagonists do differ, but this might not necessarily be manifested in the outcome of thermodynamic experiments because various contributions might cancel each other out. Appropriately chosen experimental conditions could perhaps affect the thermodynamic signatures of agonists and antagonists differently and, thus, a functional discrimination by thermodynamic experiments might become possible.

Because ΔH affords a quantitative measure of the interactions, it can contribute to the localization of enthalpy hot spots of the active site and the identification of the crucial binding motifs of ligands. Structural changes in the ligand and its protein complex that are associated with significant favorable binding enthalpy are signs of new or optimized interactions. In this way, groups responsible for these advantageous interactions can be identified [44]. Thus, these interactions might be kept in the optimization of the compounds or they could serve as templates to introduce similar interactions in other compounds.

Overcoming enthalpy–entropy compensation by designing specific new interactions is extremely difficult, making elimination of entropy-driven optimization unrealistic. Furthermore, the complexity of the binding event prevents delineating quantitative structure–thermodynamic relationships. We argue, however, that based on the evaluation of thermodynamic signatures, the practice of medicinal chemistry optimization could be thermodynamically more balanced. Basically, there are two strategies towards this goal. The first option is monitoring binding thermodynamics continuously during optimizations to support the design of thermodynamically balanced compounds in each round of the optimization cycle for follow-up [45]. The other option is the thermodynamic characterization of all available starting points and the selection of the enthalpically most favored ones for subsequent entropy-driven optimization. In addition, a combination of these strategies might also provide more viable leads. Balanced optimization can be achieved with favorable enthalpy and entropy contributions, which could give a limit on the desired entropy change. Independent of the approach used, the increasing enthalpic contributions to binding affinity would improve the quality of compounds optimized.

Case studies

In this section, we discuss the practical utility of thermodynamic characterization used in early- and late-phase optimizations. Case studies of early optimization involve both HTS-based and fragment-based approaches (renin inhibitors and carbonic anhydrase, respectively). Late-stage optimizations are exemplified by HMG-CoA and renin inhibitors.

Early-phase optimizations

Renin inhibitors

Renin is an aspartic protease of the renin–angiotensin system that cleaves its natural substrate, angiotensinogen, to angiotensin I. Angiotensin-converting enzyme processes angiotensin I further to the vasoconstrictor angiotensin II. The cleavage of angiotensinogen is the rate-determining step in the production of angiotensin II, suggesting that renin inhibitors are a promising therapy for hypertension. The efficacy of Aliskiren, the first-in-class drug, provided clinical proof of concept for the development of non-peptidic renin inhibitors. Although the first attempts to identify potent, orally active renin inhibitors were initiated almost 30 years ago, most of the peptidic or peptidomimetic compounds failed because of dissolution-limited absorption, high metabolic clearance and low oral bioavailability. Consequently, the identification and optimization of potent, non-peptidic, low MW, orally active renin inhibitors is desirable.

Diaminopyrimidine-type renin inhibitors were discovered by an HTS campaign at Pfizer identifying **1** with double-digit micromolar affinity [46] (Fig. 3). Parallel synthesis of a 450-membered focused library around the diaminopyrimidine core resulted in **2**, a low micromolar renin inhibitor. The X-ray structure of the renin-**2** complex revealed that the diaminopyrimidine part of the molecule is stabilized by five hydrogen bonds; however, this analysis identified that the large S2 hydrophobic pocket and the smaller hydrophobic S3 subpocket were unoccupied. Because preliminary studies to fill the S2 pocket failed, **2** was first tethered by a tetrahydroisoquinoline (**3**) and a benzoxazinone (**4**) ring system, which were extended by a methoxypropyl side-chain toward the S3 subpocket [47,48].

X-ray analysis of the renin-**3** complex showed that all hydrogen bonds stayed intact around the diaminopyrimidine core and that the methoxypropyl side-chain reached the S3 subpocket. Although this optimization increased the SILE significantly, there was only a small improvement in the SIHE and a marginal change in the LELP. The thermodynamic signature of **3** was recorded and compared to that of **2** (Fig. 4). New hydrophobic van der Waals contacts resulted in a moderate gain in ΔH that was less than 1 kcal/mol. Displacing ordered water molecules from the hydrophobic S3 subpocket, however, was entropically favored and compensated for the entropy loss associated with the decreased flexibility, as indicated by the almost 2 kcal/mol gain in $T\Delta S$. These significant entropy effects identify the optimization of **2** to **3** as being basically entropy driven, as indicated by the marginal improvement detected in SIHE.

Although the X-ray structure of the renin-**4** complex is not publicly available, the complex of its 2,2-dimethyl-benzoxazinone analog was crystallized and showed that all the hydrogen bonds identified for **2** exist and that the S3 subpocket was filled by the methoxypropyl side-chain. This optimization increased both SILE and SIHE significantly and largely improved LELP simultaneously. Thermodynamic profiling of **4** revealed that in addition to the entropy gain caused by filling S3, significant enthalpic components are present (Fig. 4). The methyl group in position 2 formed favored van der Waals contacts and it is probable the benzoxazinone group was involved in new polar contacts within the active site. These new interactions yielded a significant gain in enthalpy ($\Delta H \sim 4$ kcal/mol) that was only partially compensated by the entropic penalty ($T\Delta S \sim 2$ kcal/mol) caused by the decreased flexibility and desolvation entropy. The significant enthalpy effects detected here mark this optimization as being enthalpy driven, as indicated by the significant improvement detected in SIHE.

Comparing these two outcomes of the early-phase optimization, the entropically optimized compound (**3**) is somewhat more potent than that obtained by enthalpy-driven optimization (**4**). Compound **3**, however, has a significantly higher MW and higher

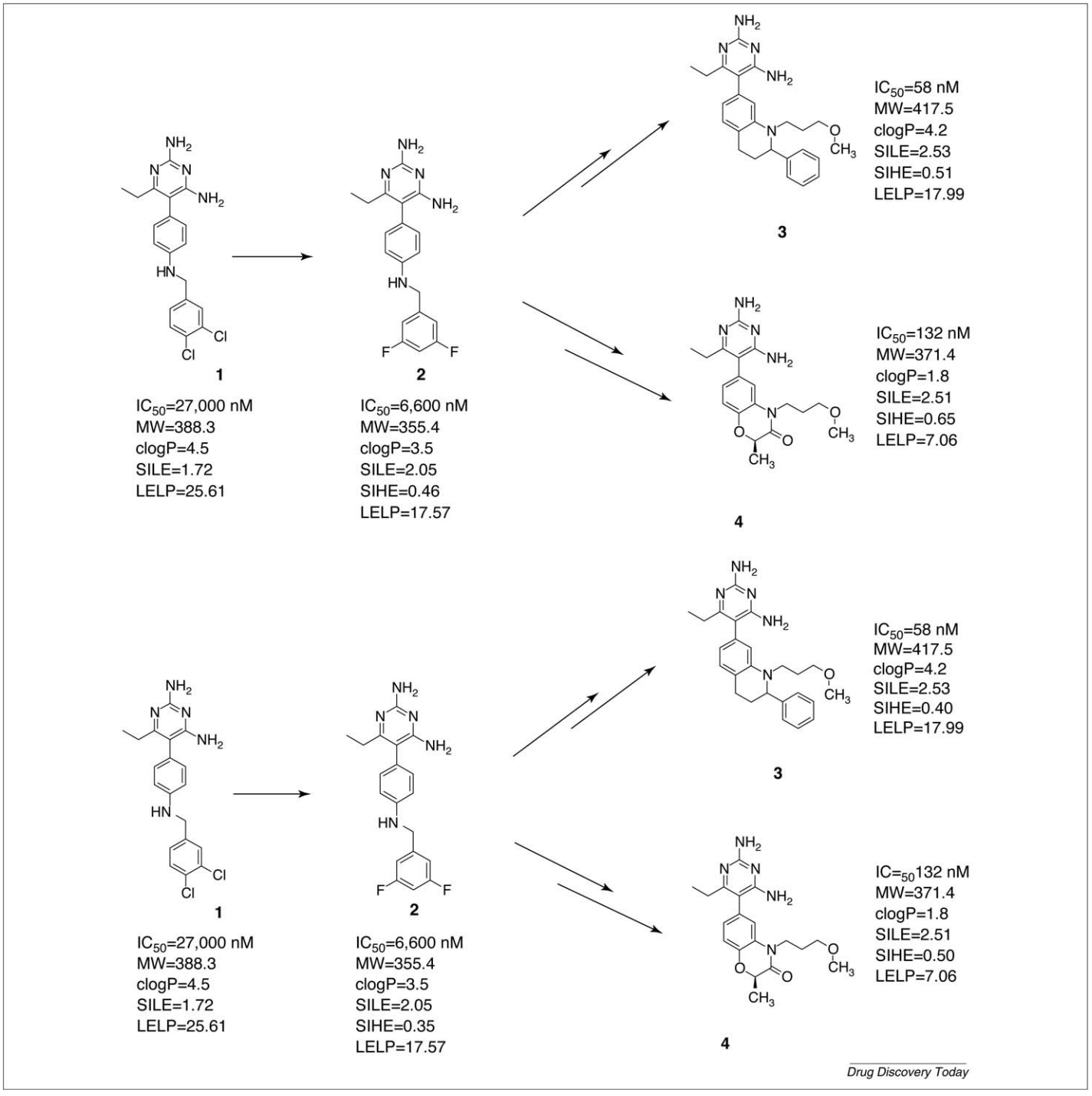


FIGURE 3

Early-phase optimization of diaminopyrimidine-type renin inhibitors.

lipophilicity than compound **4**. This finding is in line with the expectation that enthalpy-driven optimizations generate much less unfavorable shifts in physicochemical properties. SILEs are almost identical for both compounds, making the ranking difficult on this basis. The lipophilic efficiency defined by LELP [15] is, again, much better for compound **4**. Comparison of the thermodynamic signatures shows that enthalpic components are much larger for compound **4** than for compound **3,** as indicated by the corresponding SIHE values. Although the IC_{50} of compound **3** is half of that of compound **4**, physicochemical and thermodynamic data suggest the selection of compound **4** for further optimization. In fact, optimized compounds reported from these laboratories typically have benzoxazinone rather than tetrahydroisoquinoline rings [49].

Fragment-like carbonic anhydrase inhibitors

Carbonic anhydrase (CA) is a Zn-containing metallo-enzyme that catalyzes the hydration of CO_2 and the dehydration of bicarbonate. The human enzyme exists in several isoforms and is abundant in various tissues and cellular compartments. It plays a key

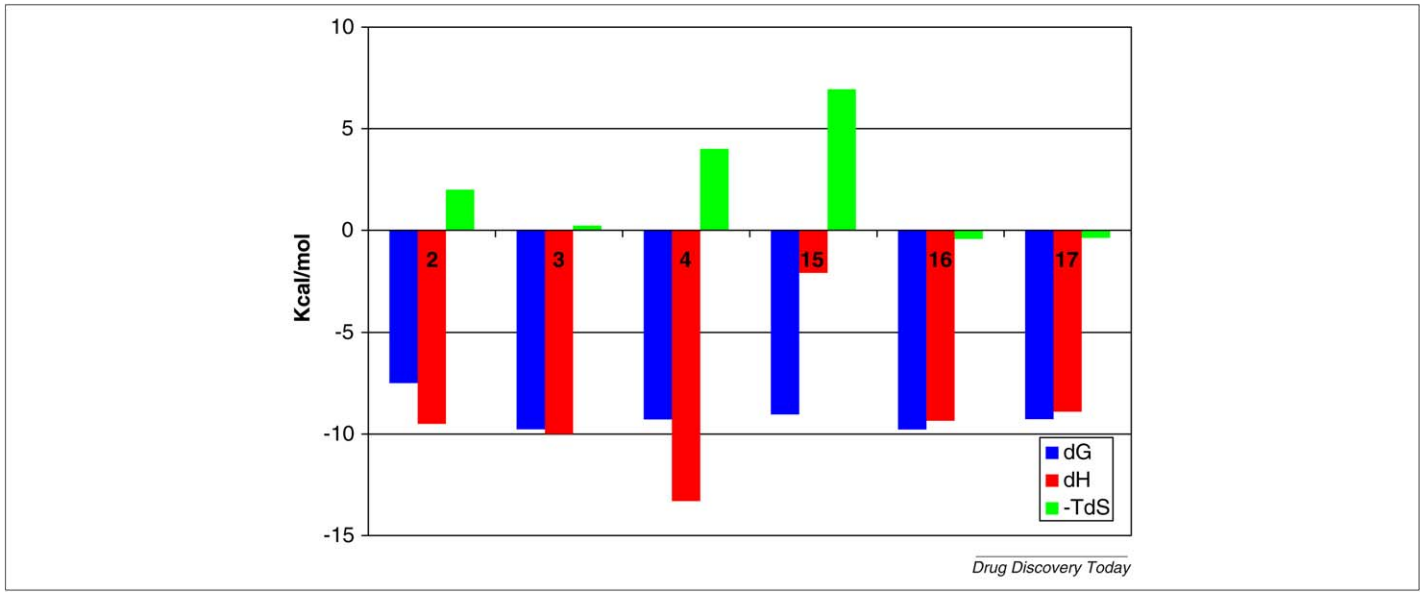


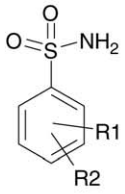
FIGURE 4

Thermodynamic profile of renin inhibitors.

part in the regulation of pH and fluid balance in different parts of the body, and its inhibitors are used in various therapies. CA inhibitors are applied as diuretics, as antiglaucoma agents, in the management of mountain sickness and for the improvement of the arterial oxygenation in chronic obstructive pulmonary disease.

CA is an ideal model system; its catalytic mechanism and structure are thoroughly studied and well characterized [35]. Furthermore, it binds benzene sulfonamides – small, fragment-like compounds (MW < 250 Da) with low flexibility and high affinity to CA. The binding of benzene sulfonamides to CA occurs without gross conformational change of the enzyme. Recently, Scott and Jones reported the thermodynamic optimization of benzene sulfonamide (BSA)-type CA fragment inhibitors supported by ITC experiments and X-ray structure determinations [50,51] (Fig. 5). Their binding to CA is dominated by the interactions of the sulfonamide group. The binding free energies have favorable enthalpy and for the majority of the compounds a smaller favorable entropy component.

Substitutions on the benzene ring result in small changes in the binding affinity and thermodynamic signature of the ligands. To make meaningful comparisons of the binding free energies and their enthalpic and entropic components, the changes in ΔG, ΔH and ΔS with respect to the unsubstituted BSA (**5**) were investigated. The $\Delta\Delta$ values enabled the tracking of subtle changes in the thermodynamics of binding (Fig. 6). *m*-Cl, *m*-CN and *m*-OMe (compounds **6**–**8**) have similar thermodynamic signatures with large negative $-\Delta(T\Delta S)$ and smaller – but still large – positive $\Delta\Delta H$ values that result in a small $\Delta\Delta G$ that is either positive or negative. The *o*-Cl compound **9** shows a basically similar thermodynamic signature by having $-\Delta(T\Delta S)$ and $\Delta\Delta H$ values with the same sign as those of the previous compounds but with lower absolute values. All four compounds have binding thermodynamics inferior to the reference BSA because the entropy content of their binding was increased at the expense of enthalpy loss. The *m*-F compound **10** is different in having a smaller favorable entropic and an almost absent enthalpic component. The *o*-F derivative **11** differs from all previously discussed compounds because it has a large favorable



Compound	R1	R2	K_d/nM	LELP	SILE	SIHE
5			839	0.49	3.05	0.27
6		*m*-Cl	591	2.30	3.03	0.19
7		*m*-OMe	1640	1.04	2.75	0.18
8		*m*-CN	423	0.38	3.02	0.22
9		*o*-Cl	1125	1.29	2.90	0.25
10		*m*-F	118	1.11	3.37	0.28
11		*o*-F	314	0.68	3.17	0.35
12	*p*-benzylamide		27	4.2	3.08	0.43
13	*p*-benzylamide	*m*-F	20	4.36	3.09	0.49
14	*p*-benzylamide	*o*-F	5.7	4.84	3.31	0.62

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FIGURE 5

Fragment-like sulfonamide-type carbonic anhydrase inhibitors.

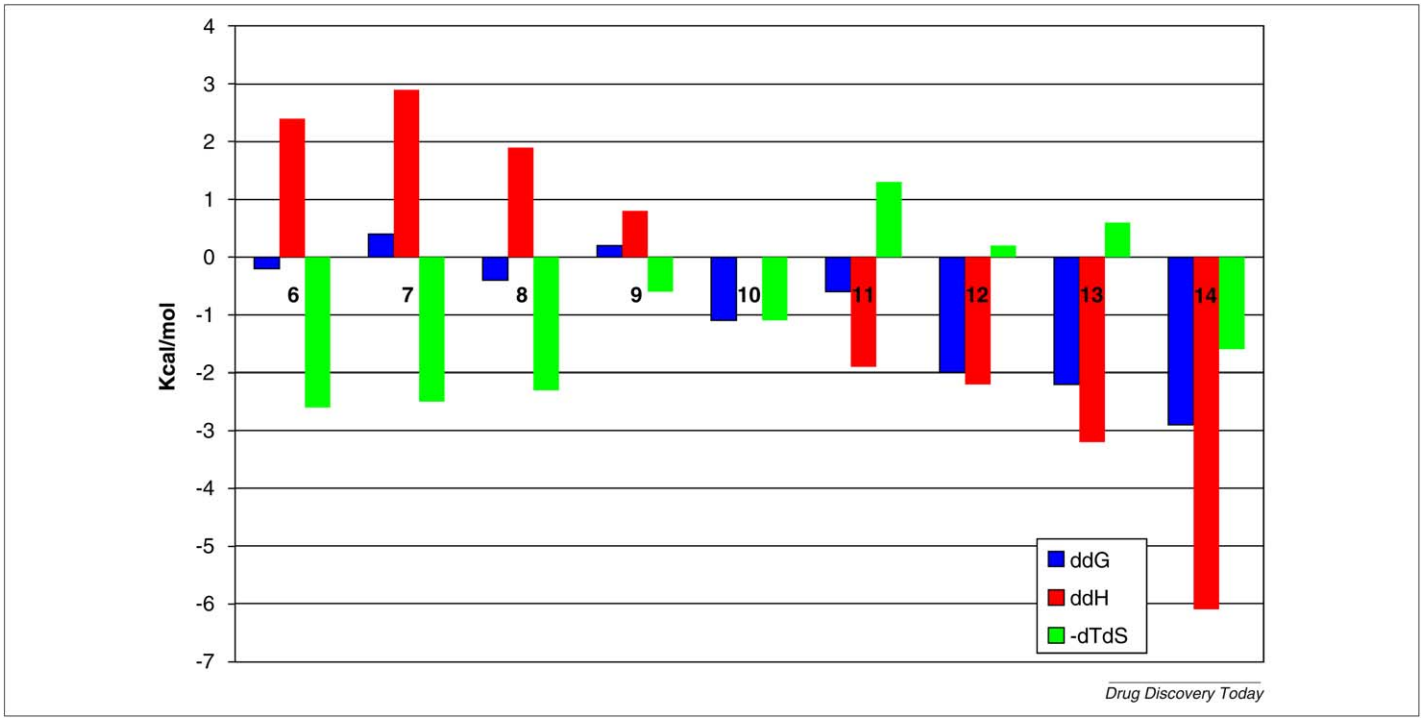


FIGURE 6

Thermodynamic profile of carbonic anhydrase inhibitors of Figure 5. $\Delta\Delta G$, $\Delta\Delta H$ and $-\Delta T\Delta S$ values relative to unsubstituted benzene sulfonamide 5.

enthalpic and a smaller, but still important, unfavorable entropic component.

An analysis of the X-ray structures of the *o*-Cl (**9**), *m*-F (**10**) and *o*-F (**11**) complexes supports the interpretation of the different thermodynamic signatures. The fluorine atom of the *o*-F derivative points towards the main chain amide NH of Thr200 and this interaction presumably contributes to the enthalpy of binding. By contrast, the fluorine atom of the *m*-F derivative points in the opposite direction towards a dominantly hydrophobic surface of the enzyme. The aromatic ring of the *o*-Cl derivative is twisted with respect to the position of fluorine derivatives and places the Cl-atom in a hydrophobic pocket. These structural differences rationalize why *m*-F and *o*-Cl derivatives have decreased enthalpy and increased entropy content and explain the privileged binding thermodynamics of the *o*-F derivative. The SILE of the BSA derivatives **5**–**11** has a maximum at the *m*-F derivative **10**, and the SIHE of these compounds has a maximum at the *o*-F BSA **11**. The high SIHE of **11** accompanied with a reasonable SILE value suggests this compound as being a more appropriate starting point for further optimization owing to its more important enthalpy component.

Compounds **12**–**14** include a *p*-benzylamide group (Fig. 5). Whereas **12** contains no further substituents, **13** and **14** are substituted by fluorine in the *meta* and *ortho* positions, to the sulfonamide group, respectively. Thus, within the pairs of **5** and **12**, **10** and **13**, and **11** and **14**, either the absence of or the position of the F-substituent relative to the sulfonamide group is the same. The addition of the benzylamide group significantly improves binding in all three pairs, but the change in the binding free energy and its enthalpy and entropy components varies. This can be attributed to the varying intra- and intermolecular interactions in these compounds and in their CA complexes. Although no experimental structure for these complexes is available, the binding features can be assumed from the X-ray structures of BSA derivatives and *para*-substituted benzylamide derivatives. The carboxamide group H-bonded to a water molecule that, in turn, is H-bonded to the enzyme. The F-substitution has a position-dependent effect on the H-bond acceptor ability of this carboxamide group. In addition, both the carboxamide and the sulfonamide groups can directly interact with an adjacent fluorine atom. Compound **14** shows the highest affinity and the highest enthalpy component, and its superiority is readily shown by its favorable SILE and SIHE values (Fig. 5). Although the most favorable enthalpy component among BSA derivatives **5**–**11** was already identified for *o*-F BSA **11**, the addition of the *p*-benzylamide group not only increased the enthalpy content of binding but also resulted in the highest affinity compound. Thus, F-substitution *ortho* to the sulfonamide group has privileged properties in the BSA series, and this suggests that *o*-F BSA derivatives are particularly well suited for further investigations. In spite of this finding, a search in Prous Science Integrity® [49] resulted in 15 *m*-F BSA derivatives associated with CA activity and no *o*-F BSA derivatives, as reported by Scott and Jones [50,51].

Late-phase optimizations

Renin inhibitors

Diaminopyrimidine-type renin inhibitors were next optimized toward the large hydrophobic S2 pocket (Fig. 7). NMR auxiliary screens identified an N-aryl-benzamide that binds to the S2 pocket. Inter-ligand nuclear Overhauser- effect (NOE) data suggested that the N-aryl-benzamide can be linked to the amino group located in position 4 of the diaminopyrimidine core. Compound **15** identified in this way showed somewhat less affinity (IC_{50} = 336 nM), probably

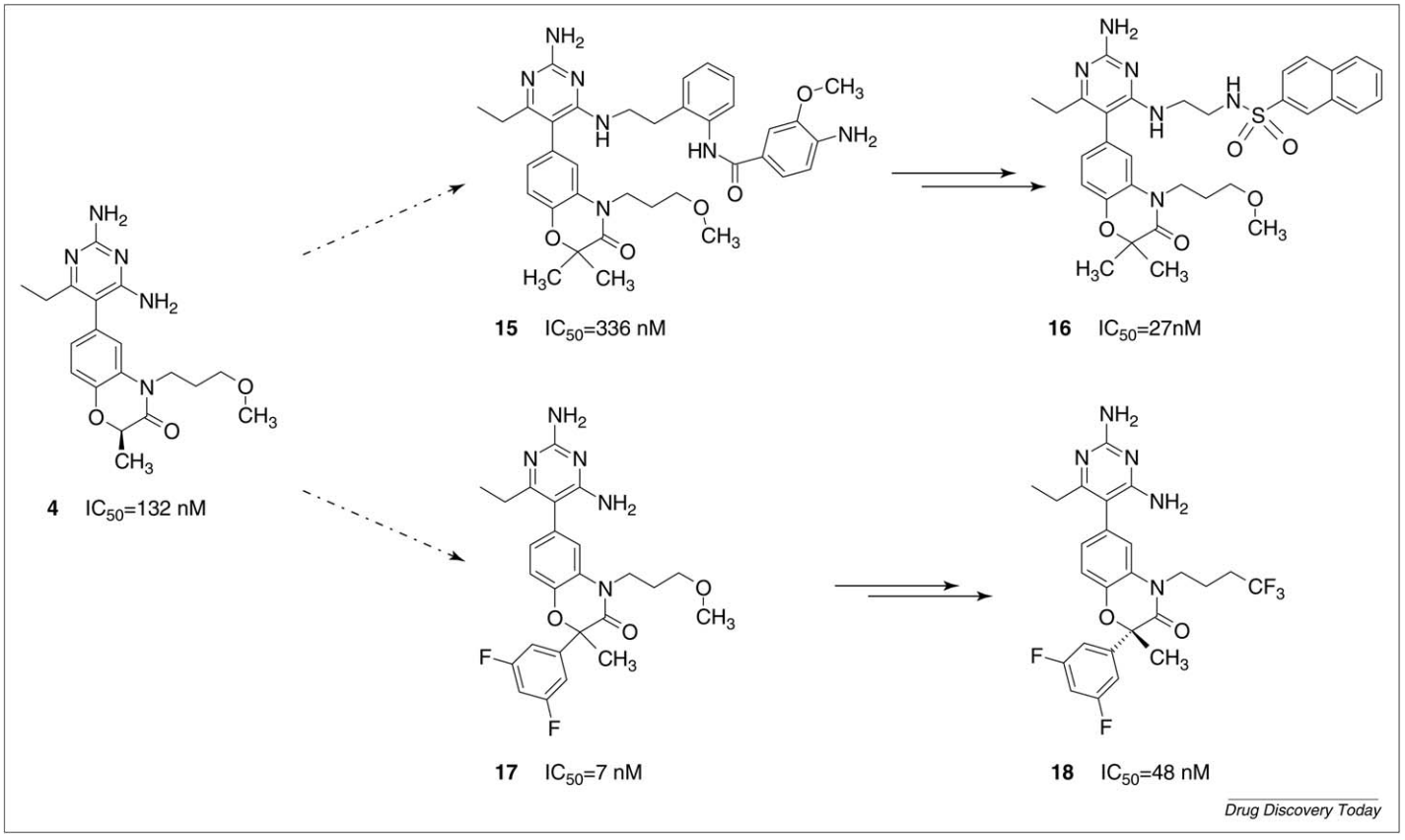


FIGURE 7

Late-phase optimization of diaminopyrimidine-type renin inhibitors.

because of the suboptimal contacts formed within the S2 pocket. Thermodynamic profiling of this compound (Fig. 4) indicated a significant loss in binding enthalpy and a large gain in entropy. Disfavored binding enthalpy suggested that polar groups in the aryl benzamide moiety could not form H-bonds in the S2 pocket and, thus, the penalty in desolvation could not be compensated by enthalpic factors. A huge gain in binding entropy could be rationalized by the displacement of ordered water molecules from the large hydrophobic S2 pocket. Based on these data, the team concluded that affinity could be improved by positioning substituents to interact with the negatively and positively polarized areas in the S2 pocket. Extensive optimization of S2 substituents led to the identification of **16**. The thermodynamic signature of this compound nicely justified its design concept (Fig. 4) because significant gain in binding enthalpy was detected; however, new polar interactions decreased the flexibility of the inhibitor and the protein backbone, resulting in a less favored binding entropy. Consequently, the binding affinity of **16** was found to be more than 10 times higher than that of compound **15**.

An alternative optimization scheme focused on the central region and the S3 subpocket. Introducing the previously identified difluorophenyl moiety into position 2 of the benzoxazinone ring resulted in **17**, with improved affinity. Although the S2 pocket remained empty in this case, the thermodynamic signature of this compound was similar to that of compound **16**. Further optimization of the side-chain that fills the S3 subpocket finally led to compound **18**, having somewhat less affinity but improved ADME properties. **18** showed good bioavailability both in rat (74%) and dog (19%), triggering its selection for preclinical development as identified from Prous Science Integrity® [49].

HMG-CoA reductase inhibitors

HMG-CoA reductase (HMGR) is an integral protein of endoplasmic reticulum membranes. HMGR catalyzes production of mevalonate from HMG-CoA, which is the rate-limiting step in cholesterol biosynthesis. HMGR inhibitors – such as statins – therefore effectively lower serum cholesterol levels. Statins prevent cardiovascular diseases and, although they are generally well tolerated, myalgia is a reported side-effect. This can be reduced by targeting hepatic tissues, and it has been shown that hydrophilic statins tend to be more hepatoselective [52].

Novel inhibitors of HMGR with improved preclinical efficacy and hepatoselectivity were sought [53]. Six series of analogs of earlier statins were investigated. They contain a central heteroaromatic ring substituted by 3,5-dihydroxyheptanoic acid and in most cases an adjacent aromatic group. Series 1 and 2 have imidazole cores and differ in the N-atom positions in the core. Series 3 contains pyrrole-based bicyclic compounds, and series 4–6 have pyrrole cores with different N positions. Fig. 8 shows the highest affinity compound from each series together with rosuvastatin (**25**), which is shown for reference.

Biochemical assay, crystallography and ITC were used to characterize the compounds, the majority of which are in the nanomolar potency range. The most potent compounds in each series

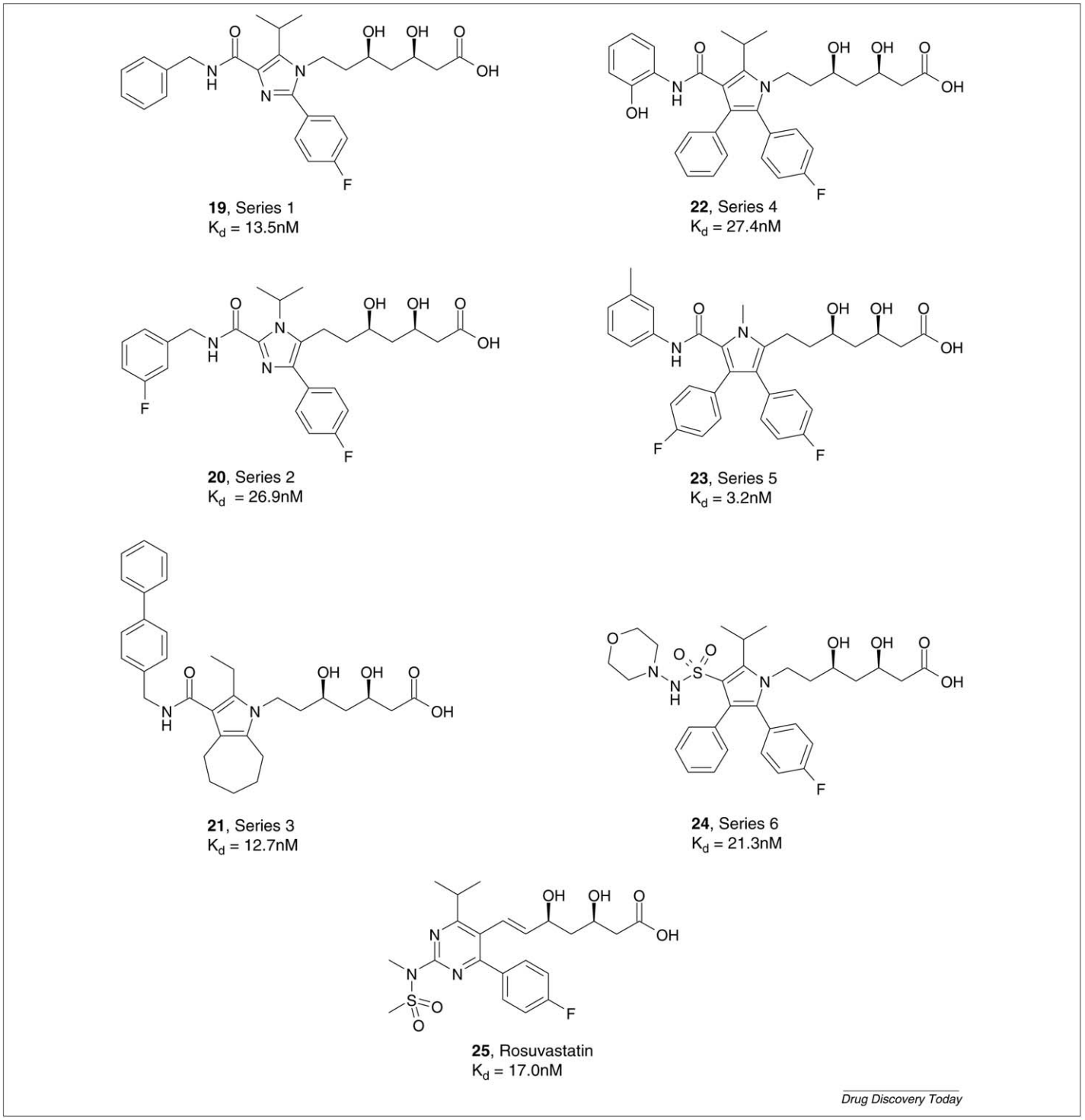


FIGURE 8

HMG-CoA reductase inhibitors. Highest affinity compounds from each series and rosuvastatin.

have single-digit nanomolar activity. By contrast, enthalpy and entropy components vary considerably among the different series, although much less variation was observed within the series. Fig. 9 shows the thermodynamic signature of the highest affinity compound from each series.

Series 1 and 2 have highly favorable binding free energy and the highest enthalpy component among all series. Unique structural features in series 1 and 2 include N-benzyl rather than N-phenyl substitution of the amide group and the unsubstituted core N-atom adjacent to the amide substituent. The authors proposed that these two factors are responsible for the favorable thermodynamic profile of these compounds. An analysis of the X-ray structure of **19** suggests that the phenyl to benzyl replacement has the advantage that the increased flexibility of the latter promotes the phenyl ring into an advantageous position and preserves the H-bond between the amide carbonyl and Ser565. These optimized

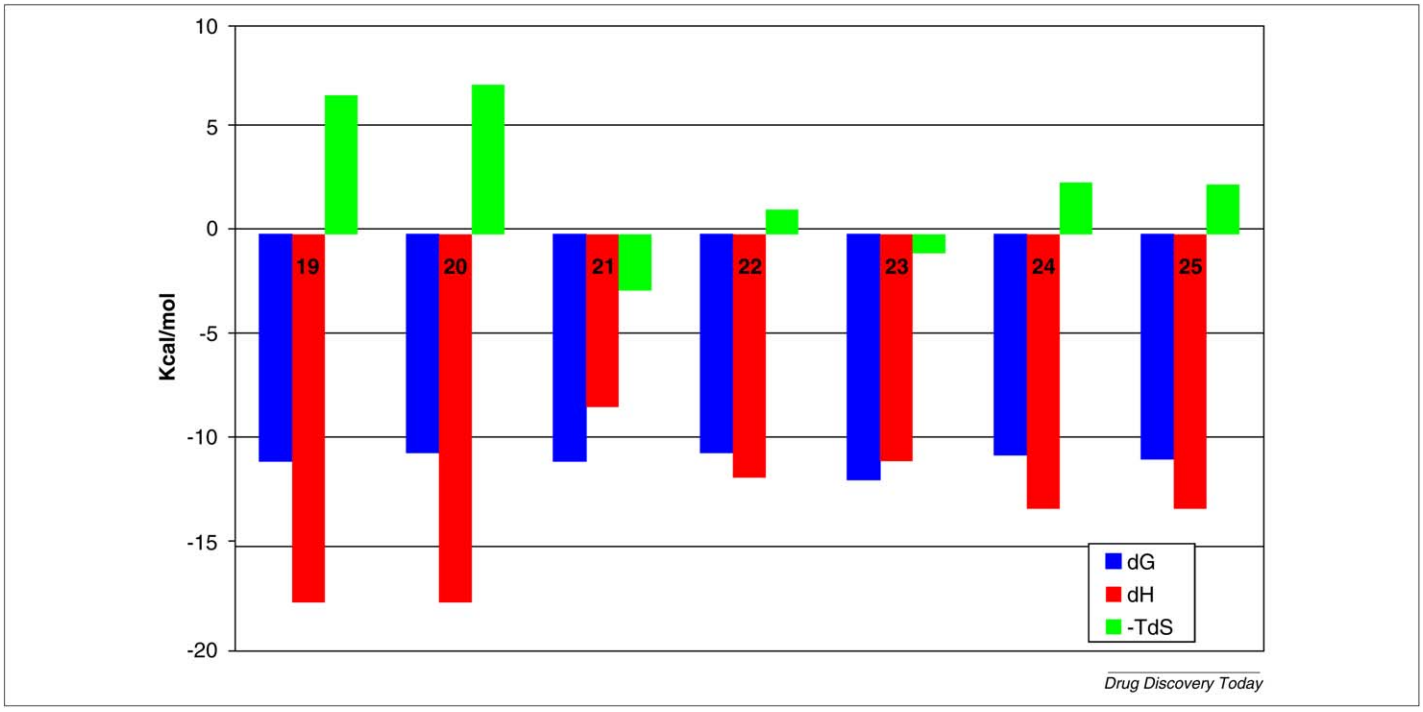


FIGURE 9

Thermodynamic profile of HMG-CoA reductase inhibitors.

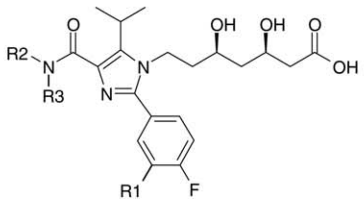
interactions contribute to an increased binding enthalpy. The absence of the substituent *ortho* to the carboxamide group is also advantageous for the thermodynamic profile. In the complexes of other series, the partially solvent exposed substituents in this position result in an entropy gain at the expense of an enthalpy loss.

Series 3 compounds have considerably lower binding enthalpy than compounds in series 1 and 2. The X-ray structure of **21** from series 3 reveals that the protein undergoes significant conformational movements that complicate the thermodynamic analysis of these complexes.

The thermodynamic signatures of series 4, 5 and 6 are also inferior to those of series 1 and 2. This is rationalized by the replacement of the preferred N-benzyl by N-phenyl substituents and by the unfavorable substitution of the core N-atom (*c.f.* analysis of series 1 and 2). Series 4 and 5 are similar in structure and in terms of binding energy components. The change in the N position is not crucial for the interactions in accordance with the observed similarity between series 1 and 2. Series 6 compounds contain a sulfonamide in position 4. Apparently, the replacement of carboxamide by sulfonamide has no significant effect on the binding, in line with the partial water exposure of these moieties.

The conclusion of this analysis is that although the different series show similar affinities, they can be distinguished by their thermodynamic profiles. The high enthalpic content of binding of series 1 and 2 favors these series. Because series 1 is more hepatoselective than series 2 [53], the former is the most appropriate starting point for further development. It is also noteworthy that series 1 has a thermodynamic signature superior to rosuvastatin that was shown to bind with the highest enthalpy content among marketed statins [21,54].

Compounds from series 1 are shown in Fig. 10, and their thermodynamic signatures are in Fig. 11. With the exception of **30,** they are characterized by high enthalpy that overcompensates for their unfavorable entropy. The best affinity was measured for **26**, **27** and **29**, although compound **19**, with slightly lower



Compound	R1	R2	R3
19	H	benzyl	H
26	F	benzyl	H
27	H	4-Ph-benzyl	H
28	H	(*S*)-CH(CH$_2$OH)Ph	H
29	H	(*S*)-CH(CH$_3$)Ph	Me
30	H	3-(2-F-Ph)-benzyl	H

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FIGURE 10

HMG-CoA reductase inhibitors of series 1.

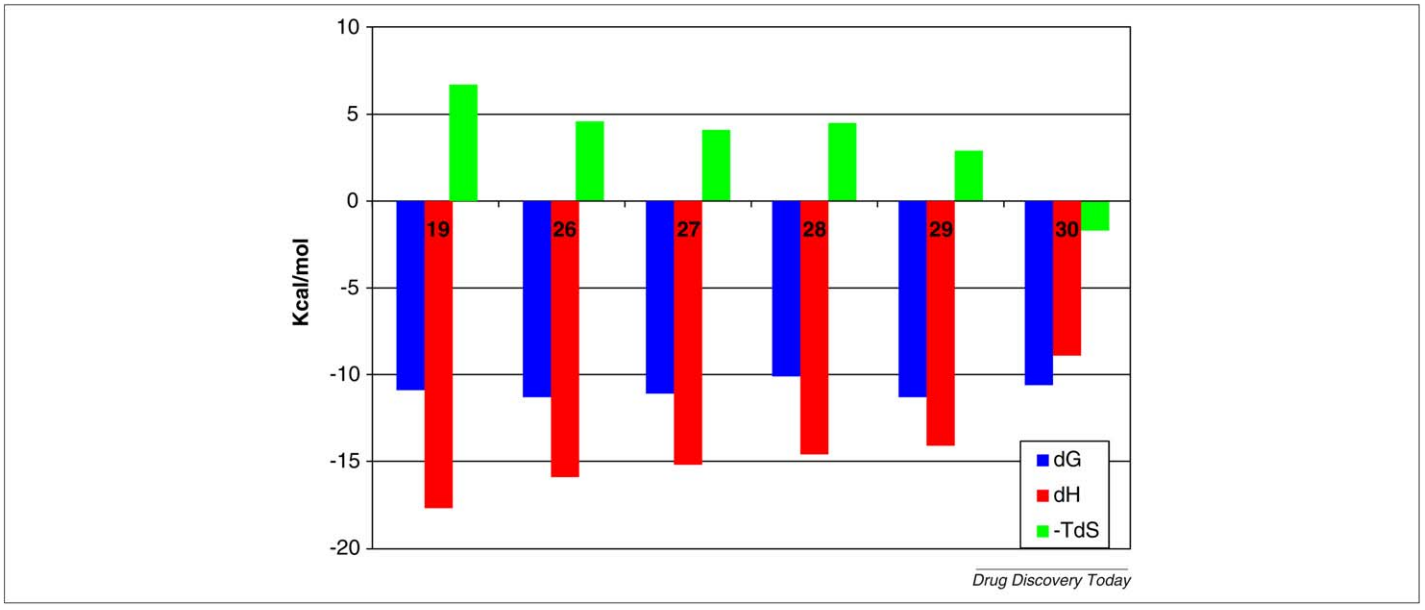

FIGURE 11

Thermodynamic profile of series 1 HMG-CoA reductase inhibitors.

affinity, has higher binding enthalpy. Owing to its advantageous balance of properties – including HMGR affinity, binding enthalpy, biological potency, and hepatoselectivity – **19** was selected for preclinical development [49,53].

Concluding remarks

Although the undesirable shift in physicochemical properties of leads was first reported more than a decade ago, the current practice of drug discovery still tends to generate complex and apolar structures that are not ideally suited for clinical development. Recently, we showed that this phenomenon could be traced back to lead discovery (i.e. hit-to-lead optimization is responsible for an unfavorable shift of physicochemical properties). Comparing fragment and HTS hit and lead pairs screened against exactly the same set of targets, we demonstrated that fragment-based optimizations are also affected. Because unfavorable changes in properties seem to be independent of the lead discovery technology applied, we hypothesized that the optimization strategy should have a major impact on compound quality. Analyzing the thermodynamic basis of affinity optimizations, we concluded that entropy-driven optimization strategies contribute significantly to this undesired trend.

We argue that thermodynamically more balanced strategies might provide better quality leads and clinical candidates. Comparative thermodynamic analysis of lead candidates could help identify enthalpically favored starting points. Monitoring thermodynamic profiles along optimization pathways might enable a proper balance between entropic and enthalpic contributions. Promoting enthalpic optimizations, we introduce a size-independent measure of enthalpic efficiency (SIHE) that makes unbiased comparison of leads and the progression of compounds in discovery programs possible. Early case studies discussed here show that the concept of thermodynamics guided lead discovery and optimization could contribute to the success of both fragment-based and traditional medicinal chemistry programs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.drudis.2010.08.013.

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